



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> : C12N 15/12, C12P 21/02 A61K 37/02		A1	(11) International Publication Number: WO 93/00432
			(43) International Publication Date: 7 January 1993 (07.01.93)
(21) International Application Number: PCT/US92/05374		(74) Agent: KAPINOS, Ellen, J.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).	
(22) International Filing Date: 25 June 1992 (25.06.92)		(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).	
(30) Priority data: 720,590 25 June 1991 (25.06.91) US		Published With international search report.	
(71) Applicant (for all designated States except US): GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only) : WOZNEY, John, M. [US/US]; 59 Old Bolton Road, Hudson, MA 01749 (US). CELESTE, Anthony, J. [US/US]; 86 Parkard Street, Hudson, MA 01749 (US).			

## (54) Title: BMP-9 COMPOSITIONS

```

TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG      48
  * Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                               -35                -30

CAG GTG AGA GCA GTC ACG AGG AGG ACA CCG ATG GCG CAC GTG GCT GCG      96
Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                               -20                -15                -10

GGG TCG ACT TTA GCC AGG CCG AAA AGG AGC GCC GGG GCT GGC AGC CAC      144
Gly Ser Thr Leu Ala Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                               -5                        1                        5

TGT CAA AAG ACC TCC CTG CCG GTA AAC TTC GAG GAC ATC GGC TGG GAC      192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
                               10                        15                        20

AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC      240
Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
                               25                        30                        35

GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT      288
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
40                               45                        50                        55

ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG      336
Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                               60                        65                        70

GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG      384
Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
75                               80                        85

GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC      432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
90                               95                        100

CTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG      470
Val Ala Glu Cys Gly Cys Arg
105                               110

```

## (57) Abstract

Purified BMP-9 proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and cartilage defects and in wound healing and related tissue repair.

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MI	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TC	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

5

## BMP-9 COMPOSITIONS

10 The present invention relates to a novel family of purified proteins designated BMP-9 proteins and processes for obtaining them. These proteins may be used to induce bone and/or cartilage formation and in wound healing and tissue repair.

15 The murine BMP-9 DNA sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2) are set forth in Figure 1. Human BMP-9 sequence is set forth in Figure 3 (SEQ ID NO: 8 and SEQ ID NO: 9). It is contemplated that BMP-9 proteins are capable of inducing the formation of cartilage and/or bone. BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat  
20 bone formation assay described below.

Murine BMP-9 is characterized by comprising amino acid #319 to #428 of Figure 1 (SEQ ID NO: 2 amino acids #1-110). Murine BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #610 to nucleotide  
25 #1893 as shown in Figure 1 (SEQ ID NO: 1) and recovering and purifying from the culture medium a protein characterized by the amino acid sequence comprising amino acid #319 to #428 as shown in Figure 1 (SEQ ID NO: 2) substantially free from other proteinaceous materials with which it is co-produced.

30 Human BMP-9 is expected to be homologous to murine BMP-9 and is characterized by comprising amino acid #1 (Ser, Ala, Gly) to #110 of Figure 3 (SEQ ID NO: 9) (Arg). The invention includes methods for obtaining the DNA sequences encoding human BMP-9. This method entails utilizing the murine BMP-9  
35 nucleotide sequence or portions thereof to design probes to screen libraries for the human gene or fragments thereof using standard techniques. Human BMP-9 may be produced by culturing

a cell transformed with the BMP-9 DNA sequence and recovering and purifying BMP-9 from the culture medium. The expressed protein is isolated, recovered, and purified from the culture medium. The purified expressed protein is substantially free from other proteinaceous materials with which it is co-produced, as well as from other contaminants. The recovered purified protein is contemplated to exhibit cartilage and/or bone formation activity. The proteins of the invention may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO: 8 and recovering and purifying from the culture medium a protein characterized by the amino acid sequence of SEQ ID NO: 9 from amino acid #1 to amino acid #110 substantially free from other proteinaceous materials with which it is co-produced.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of a BMP-9 protein in a pharmaceutically acceptable vehicle or carrier. BMP-9 compositions of the invention may be used in the formation of cartilage. These compositions may further be utilized for the formation of bone. BMP-9 compositions may also be used for wound healing and tissue repair. Compositions of the invention may further include at least one other therapeutically useful agent such as the BMP proteins BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, and BMP-7 disclosed for instance in PCT publications WO88/00205, WO89/10409, and WO90/11366, and BMP-8, disclosed in U.S. application Ser. No. 07/641,204 filed January 15, 1991, Ser. No. 07/525,357 filed May 16, 1990, and Ser. No. 07/800,364 filed November 20, 1991.

The compositions of the invention may comprise, in addition to a BMP-9 protein, other therapeutically useful agents including growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth

factor (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF). The compositions may also include an appropriate matrix for instance, for supporting the composition and providing a surface for bone and/or cartilage growth. The matrix may provide slow release of the osteoinductive protein and/or the appropriate environment for presentation thereof.

The BMP-9 compositions may be employed in methods for treating a number of bone and/or cartilage defects, periodontal disease and various types of wounds. These methods, according to the invention, entail administering to a patient needing such bone and/or cartilage formation wound healing or tissue repair, an effective amount of a BMP-9 protein. These methods may also entail the administration of a protein of the invention in conjunction with at least one of the novel BMP proteins disclosed in the co-owned applications described above. In addition, these methods may also include the administration of a BMP-9 protein with other growth factors including EGF, FGF, TGF- $\alpha$ , TGF- $\beta$ , and IGF.

Still a further aspect of the invention are DNA sequences coding for expression of a BMP-9 protein. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Figure 1 (SEQ ID NO: 1) and Figure 3 (SEQ ID NO: 8) or DNA sequences which hybridize under stringent conditions with the DNA sequences of Figure 1 or 3 and encode a protein having the ability to induce the formation of cartilage and/or bone. Finally, allelic or other variations of the sequences of Figure 1 or 3, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present invention.

A further aspect of the invention includes vectors comprising a DNA sequence as described above in operative association with an expression control sequence therefor. These vectors may be employed in a novel process for producing a BMP-9 protein of the invention in which a cell line transformed with a DNA sequence encoding a BMP-9 protein in

operative association with an expression control sequence therefor, is cultured in a suitable culture medium and a BMP-9 protein is recovered and purified therefrom. This process may employ a number of known cells both prokaryotic and eukaryotic as host cells for expression of the polypeptide.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

#### Brief Description of the Drawing

FIG. 1 comprises DNA sequence and derived amino acid sequence of murine BMP-9 from clone ML14a further described below.

FIG. 2 comprises DNA sequence and derived amino acid sequence of human BMP-4 from lambda U2OS-3 ATCC #40342.

FIG. 3 comprises DNA sequence and derived amino acid sequence of human BMP-9 from  $\lambda$  FIX/H6111 ATCC # 75252.

#### Detailed Description of the Invention

The murine BMP-9 nucleotide sequence (SEQ ID NO: 1) and encoded amino acid sequence (SEQ ID NO: 2) are depicted in Figure 1. Purified murine BMP-9 proteins of the present invention are produced by culturing a host cell transformed with a DNA sequence comprising the DNA coding sequence of Figure 1 (SEQ ID NO: 1) from nucleotide #610 to nucleotide #1893 and recovering and purifying from the culture medium a protein which contains the amino acid sequence or a substantially homologous sequence as represented by amino acid #319 to #428 of Figure 1 (SEQ ID NO: 2). The BMP-9 proteins recovered from the culture medium are purified by isolating them from other proteinaceous materials from which they are co-produced and from other contaminants present.

Human BMP-9 nucleotide and amino acid sequence is depicted in SEQ ID No: 8 and 9. Mature human BMP-9 is expected to comprise amino acid #1 (Ser, Ala, Gly) to #110 (Arg).

Human BMP-9 may be produced by culturing a cell transformed with a DNA sequence comprising nucleotide #124 to #453 as shown in SEQ ID NO: 8 and recovering and purifying from the culture medium a protein characterized by the amino acid sequence of SEQ ID NO: 9 from amino acid #1 to amino acid #110 substantially free from other proteinaceous materials with which it is co-produced.

BMP-9 proteins may be characterized by the ability to induce the formation of cartilage. BMP-9 proteins may be further characterized by the ability to induce the formation of bone. BMP-9 proteins may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described below.

The BMP-9 proteins provided herein also include factors encoded by the sequences similar to those of Figure 1 and 3 (SEQ ID NO's: 1 and 8), but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Figure 1 of Figure 3 (SEQ ID NO's: 2 and 9). These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Figure 1 and Figure 3 may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring BMP-9 and other BMP-9 polypeptides in therapeutic processes.

Other specific mutations of the sequences of BMP-9 proteins described herein involve modifications of glycosylation sites. These modifications may involve O-linked

or N-linked glycosylation sites. For instance, the absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at asparagine-linked glycosylation recognition sites. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for BMP-9 proteins. These DNA sequences include those depicted in Figure 1 or Figure 3 (SEQ ID NO's: 1 and 8) in a 5' to 3' direction and those sequences which hybridize thereto under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] and encode a protein having cartilage and/or bone inducing activity.

Similarly, DNA sequences which code for BMP-9 proteins coded for by the sequences of Figure 1 or Figure 3, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel factors described herein. Variations in the DNA sequences of Figure 1 or Figure 3 (SEQ ID NO: 1 and 8) which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded are also encompassed in the invention.

Another aspect of the present invention provides a novel



method for producing BMP-9 proteins. The method of the present invention involves culturing a suitable cell line, which has been transformed with a DNA sequence encoding a BMP-9 protein of the invention, under the control of known regulatory sequences. The transformed host cells are cultured and the BMP-9 proteins recovered and purified from the culture medium. The purified proteins are substantially free from other proteins with which they are co-produced as well as from other contaminants.

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening, product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. The mammalian cell CV-1 may also be suitable.

Bacterial cells may also be suitable hosts. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel BMP-9 polypeptides. Preferably the vectors contain the full novel

DNA sequences described above which encode the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the BMP-9 protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present invention. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Regulatory sequences for such vectors are known to those skilled in the art and may be selected depending upon the host cells. Such selection is routine and does not form part of the present invention.

A protein of the present invention, which induces cartilage and/or bone formation in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a BMP-9 protein may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. A BMP-9 protein may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. BMP-9 polypeptides of the invention may also be useful in the treatment of osteoporosis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

35

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair).

It is further contemplated that proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease in neuronal survival.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. The invention further comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of at least one of the BMP-9 proteins of the invention in admixture with a pharmaceutically acceptable vehicle, carrier or matrix.

It is expected that the proteins of the invention may act in concert with or perhaps synergistically with other related proteins and growth factors. Further therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of at least one BMP-9 protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned applications described above. Such combinations may comprise separate molecules of the BMP proteins or heteromolecules comprised of different BMP moieties. For example, a method and composition of the invention may comprise a disulfide linked dimer comprising a BMP-9 protein subunit and a subunit from one of the "BMP" proteins described above. A further embodiment may comprise a heterodimer of BMP-9 moieties. Further, BMP-9 proteins may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF),

transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH,  
5 isotonicity, stability and the like, is within the skill of the art. The therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients  
10 for such treatment with BMP-9 of the present invention.

The therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free,  
15 physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the  
20 BMP-9 proteins which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the BMP composition in the methods of the invention.

Preferably for bone and/or cartilage formation, the  
25 composition would include a matrix capable of delivering BMP-9 or other BMP proteins to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. The matrix may provide slow release of BMP-9 and/or the  
30 appropriate environment for presentation thereof. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties,  
35 cosmetic appearance and interface properties. The particular

application of the BMP-9 compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, 5 polylactic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as 10 sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium- 15 aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the BMP-9 protein, e.g. amount of bone weight desired to be 20 formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and 25 the types of BMP proteins in the composition. The addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, x-rays, 30 histomorphometric determinations and tetracycline labeling.

The following examples illustrate practice of the present invention in recovering and characterizing murine BMP-9 protein and employing it to recover the human and other BMP-9 proteins, obtaining the human proteins and expressing the proteins via 35 recombinant techniques.

## EXAMPLE I

Murine BMP-9

750,000 recombinants of a mouse liver cDNA library made in the vector lambdaZAP (Stratagene/Catalog #935302) are plated and duplicate nitrocellulose replicas made. A fragment of human BMP-4 DNA corresponding to nucleotides 1330-1627 of Figure 2 (SEQ ID NO: 3) (the human BMP-4 sequence) is <sup>32</sup>p-labeled by the random priming procedure of Feinberg et al. [Anal. Biochem. 132: 6-13 (1983)] and hybridized to both sets of filters in SHB at 60°C for 2 to 3 days. Both sets of filters are washed under reduced stringency conditions (4X SSC, 0.1% SDS at 60°C). Many duplicate hybridizing recombinants of various intensities (approximately 92) are noted. 50 of the strongest hybridizing recombinant bacteriophage are plaque purified and their inserts are transferred to the plasmid Bluescript SK (+/-) according to the in vivo excision protocol described by the manufacturer (Stratagene). DNA sequence analysis of several recombinants indicate that they encode a protein homologous to other BMP proteins and other proteins in the TGF- $\beta$  family. The DNA sequence and derived amino acid sequence of one recombinant, designated ML14a, is set forth in Figure 1. (SEQ ID NO: 1)

The nucleotide sequence of clone ML14a contains an open reading frame of 1284 bp, encoding a BMP-9 protein of 428 amino acids. The encoded 428 amino acid BMP-9 protein is contemplated to be the primary translation product as the coding sequence is preceded by 609 bp of 5' untranslated sequence with stop codons in all three reading frames. The 428 amino acid sequence predicts a BMP-9 protein with a molecular weight of 48,000 daltons.

Based on knowledge of other BMP proteins and other proteins within the TGF- $\beta$  family, it is predicted that the precursor polypeptide would be cleaved at the multibasic sequence ARG-ARG-LYS-ARG in agreement with a proposed consensus

proteolytic processing sequence of ARG-X-X-ARG. Cleavage of the BMP-9 precursor polypeptide at this location would generate a 110 amino acid mature peptide beginning with the amino acid SER at position #319. The processing of BMP-9 into the mature form is expected to involve dimerization and removal of the N-terminal region in a manner analogous to the processing of the related protein TGF- $\beta$  [L.E. Gentry, et al., Molec. & Cell. Biol. 8:4162 (1988); R. Derynck, et al., Nature 316:701 (1985)].

It is contemplated therefore that the mature active species of murine BMP-9 comprises a homodimer of 2 polypeptide subunits, each subunit comprising amino acids #319-#428 with a predicted molecular weight of approximately 12,000 daltons. Further active species are contemplated comprising amino acids #326 - #428 thereby including the first conserved cysteine residue. As with other members of the BMP and TGF- $\beta$  family of proteins, the carboxy-terminal region of the BMP-9 protein exhibits greater sequence conservation than the more amino-terminal portion. The percent amino acid identity of the murine BMP-9 protein in the cysteine-rich C-terminal domain (amino acids #326 - #428) to the corresponding region of other human BMP proteins and other proteins within the TGF- $\beta$  family is as follows: BMP-2, 53%; BMP-3, 43%; BMP-4, 53%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; Vgl, 50%; GDF-1, 43%; TGF- $\beta$ 1, 32%; TGF- $\beta$ 2, 34%; TGF- $\beta$ 3, 34%; inhibin  $\beta$ (B), 34%; and inhibin  $\beta$ (A), 42%.

#### EXAMPLE II

##### Human BMP-9

Murine and human osteoinductive factor genes are presumed to be significantly homologous, therefore the murine coding sequence or a portion thereof is used as a probe to screen a human genomic library or as a probe to identify a human cell line or tissue which synthesizes the analogous human cartilage and/or bone protein. A human genomic library (Toole et al., supra) may be screened with such a probe, and presumptive

positives isolated and DNA sequence obtained. Evidence that this recombinant encodes a portion of the human BMP-9 relies on the murine/human protein and gene structure homologies.

Once a recombinant bacteriophage containing DNA encoding portion of the human cartilage and/or bone inductive factor molecule is obtained, the human coding sequence can be used as a probe to identify a human cell line or tissue which synthesizes BMP-9. Alternatively, the murine coding sequence can be used as a probe to identify such human cell line or tissue. Briefly described, RNA is extracted from a selected cell or tissue source and either electrophoresed on a formaldehyde agarose gel and transferred to nitrocellulose, or reacted with formaldehyde and spotted on nitrocellulose directly. The nitrocellulose is then hybridized to a probe derived from a coding sequence of the murine or human BMP-9. mRNA is selected by oligo (dT) cellulose chromatography and cDNA is synthesized and cloned in lambda gt10 or lambda ZAP by established techniques (Toole et al., supra).

Additional methods known to those skilled in the art may be used to isolate the human and other species' BMP-9 proteins of the invention.

#### A. Isolation of Human BMP-9 DNA

One million recombinants of a human genomic library constructed in the vector  $\lambda$ FIX (Stratagene catalog # 944201) are plated and duplicate nitrocellulose replicas made. Two oligonucleotide probes designed on the basis of nucleotides #1665-#1704 and #1837-#1876 of the sequence set forth in Figure 1 (SEQ ID NO:1) are synthesized on an automated DNA synthesizer. The sequence of these two oligonucleotides is indicated below:

#1: CTATGAGTGTAAGGGGGTTGCTTCTTCCCATTGGCTGAT

#2: GTGCCAACCCCTCAAGTACCACTATGAGGGGATGAGTGTGG

These two oligonucleotide probes are radioactively labeled with



15

$\gamma^{32}\text{P}$ -ATP and each is hybridized to one set of the duplicate nitrocellulose replicas in SHB at 65°C and washed with 1X SSC, 0.1% SDS at 65°C. Three recombinants which hybridize to both oligonucleotide probes are noted. All three positively hybridizing recombinants are plaque purified, bacteriophage plate stocks are prepared and bacteriophage DNA is isolated from each. The oligonucleotide hybridizing regions of one of these recombinants, designated HG111, is localized to a 1.2 kb Pst I/Xba I fragment. This fragment is subcloned into a plasmid vector (pGEM-3) and DNA sequence analysis is performed. HG111 was deposited with the ATCC, 12301 Parklawn Drive, Rockville, Maryland USA on June 16, 1992 under the requirements of the Budapest Treaty and designated as ATCC # 75252. This subclone is designated pGEM-111. A portion of the DNA sequence of clone pGEM-111 is set forth in Figure 3 (SEQ ID NO:8/ HUMAN BMP-9 sequence). This sequence encodes the entire mature region of human BMP-9 and a portion of the propeptide. It should be noted that this sequence consists of preliminary data. Particularly, the propeptide region is subject to further analysis and characterization. For example, nucleotides #1 through #3 (TGA) encode a translational stop which may be incorrect due to the preliminary nature of the sequence. It is predicted that additional sequences present in both pGEM-111 (the 1.2 kb PstI/XbaI fragment of HG111 subcloned into pGEM) and HG111 encode additional amino acids of the human BMP-9 propeptide region. Based on knowledge of other BMPs and other proteins within the TGF- $\beta$  family, it is predicted that the precursor polypeptide would be cleaved at the multibasic sequence ARG-ARG-LYS-ARG (amino acids # -4 through # -1 of SEQUENCE ID NO:9) in agreement with a proposed consensus proteolytic processing sequence ARG-X-X-ARG. Cleavage of the human BMP-9 precursor polypeptide at this location would generate a 110 amino acid mature peptide beginning with the amino acid SER at position #1 of SEQUENCE ID NO:9 (encoded by

SUBSTITUTE SHEET

nucleotides #124 through #126 of SEQUENCE ID NO:8). The processing of human BMP-9 into the mature form is expected to involve dimerization and removal of the N-terminal region in a manner analogous to the processing of the related protein TGF- $\beta$  [L.E. Gentry, et al., Molec. & Cell. Biol. 8:4162 (1988); R. Derynck, et al., Nature 316:701 (1985)].

It is contemplated therefore that the mature active species of human BMP-9 comprises a homodimer of two polypeptide subunits, each subunit comprising amino acids #1 through #110 of SEQUENCE ID NO:9, with a predicted molecular weight of 12,000 daltons. Further active species are contemplated comprising amino acids #8 through #110 thereby including the first conserved cysteine residue. As with other members of the BMP and TGF- $\beta$  family of proteins, the carboxy-terminal portion of the human BMP-9 sequence exhibits greater sequence conservation than the amino-terminal portion. the percent amino acid identity of the human BMP-9 protein in the cysteine-rich C-terminal domain (amino acids #8 through #110) to the corresponding region of other human BMP proteins and other proteins within the TGF- $\beta$  family is as follows: BMP-2, 52%; BMP-3, 40%; BMP-4, 52%; BMP-5, 55%; BMP-6, 55%; BMP-7, 53%; murine BMP-9, 97%; Vgl, 50%; GDF-1, 44%; TGF- $\beta$ 1, 32%; TGF- $\beta$ 2, 32%; TGF- $\beta$ 3, 32%; inhibin  $\beta$  (B), 35%; and inhibin  $\beta$  (A), 41%.

### 25 EXAMPLE III

#### Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of the BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved

in 0.1 % TFA, and the resulting solution added to 20mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 1 $\mu$ m glycolmethacrylate sections are stained with Von Kossa and acid fuchsin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone. In a modified scoring method, three non-adjacent sections are evaluated from each implant and averaged. "+/-" indicates tentative identification of cartilage or bone; "+1" indicates >10% of each section being new cartilage or bone; "+2", >25%; "+3", >50%; "+4", >75%; "+5", >80%. A "-" indicates that the implant is not recovered.

It is contemplated that the dose response nature of the BMP-9 containing samples of the matrix samples will demonstrate that the amount of bone and/or cartilage formed increases with the amount of BMP-9 in the sample. It is contemplated that the control samples will not result in any bone and/or cartilage formation.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the

space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing followed by autoradiography. The activity is correlated with the protein bands and pI. To estimate the purity of the protein in a particular fraction an extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and the protein is run on SDS PAGE followed by silver staining or radioiodination and autoradiography.

#### 10 EXAMPLE IV

##### Expression of BMP-9

In order to produce murine, human or other mammalian BMP-9 proteins, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. The preferred expression system for biologically active recombinant human BMP-9 is contemplated to be stably transformed mammalian cells.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Figure 1 (SEQ ID NO: 1) or Figure 3 (SEQ ID NO: 8), or other DNA sequences encoding BMP-9 proteins or other modified sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)], pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)] and pMT2 CXM.

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) (Wong et al., Science 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of

the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

5        Plasmid pMT2 CXM is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and  
10        used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga, et al., Biotechnology 84: 636 (1984). This removes bases 1075 to 1145 relative to the Hind III site  
15        near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

5' PO-CATGGGCAGCTCGAG-3' (SEQ ID NO: 5)

at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease Xho I. A derivative of  
20        pMT2CXM, termed pMT23, contains recognition sites for the restriction endonucleases PstI, Eco RI, SalI and XhoI. Plasmid pMT2 CXM and pMT23 DNA may be prepared by conventional methods.

pEMC2b1 derived from pMT21 may also be suitable in practice of the invention. pMT21 is derived from pMT2 which is  
25        derived from pMT2-VWF. As described above EcoRI digestion excises the cDNA insert present in pMT-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. Coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

30        pMT21 is derived from pMT2 through the following two modifications. First, 76 bp of the 5' untranslated region of the DHFR cDNA including a stretch of 19 G residues from G/C tailing for cDNA cloning is deleted. In this process, a XhoI site is inserted to obtain the following sequence immediately  
35



signal and the adenovirus VA I gene, DHFR and  $\beta$ -lactamase markers and an EMC sequence, in appropriate relationships to direct the high level expression of the desired cDNA in mammalian cells.

5           The construction of vectors may involve modification of the BMP-9 DNA sequences. For instance, BMP-9 cDNA can be modified by removing the non-coding nucleotides on the 5' and 3' ends of the coding region. The deleted non-coding nucleotides may or may not be replaced by other sequences known  
10           to be beneficial for expression. These vectors are transformed into appropriate host cells for expression of BMP-9 proteins.

          One skilled in the art can manipulate the sequences of Figure 1 or Figure 3 (SEQ ID NO: 1 and 8) by eliminating or replacing the mammalian regulatory sequences flanking the  
15           coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For example, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering  
20           nucleotides therein by other known techniques). The modified BMP-9 coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be  
25           transformed into bacterial host cells and a BMP-9 protein expressed thereby. For a strategy for producing extracellular expression of BMP-9 proteins in bacterial cells, see, e.g. European patent application EPA 177,343.

          Similar manipulations can be performed for the  
30           construction of an insect vector [See, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the  
35           present invention by yeast cells. [See, e.g., procedures

described in published PCT application WO86/00639 and European patent application EPA 123,289].

5 A method for producing high levels of a BMP-9 protein of the invention in mammalian cells may involve the construction of cells containing multiple copies of the heterologous BMP-9 gene. The heterologous gene is linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This approach can be employed with a number of different cell types.

15 For example, a plasmid containing a DNA sequence for a BMP-9 of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A)3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by various methods including calcium phosphate coprecipitation and transfection, electroporation or protoplast fusion. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (e.g. sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 25 5:1750 (1983). Transformants are cloned, and biologically active BMP-9 expression is monitored by the Rosen-modified Sampath - Reddi rat bone formation assay described above in Example III. BMP-9 expression should increase with increasing levels of MTX resistance. BMP-9 polypeptides are characterized using standard techniques known in the art such as pulse labeling with [35S] methionine or cysteine and polyacrylamide gel electrophoresis. Similar procedures can be followed to produce other related BMP-9 proteins.



#### A. BMP-9 Vector Construction

In order to produce human BMP-9 proteins of the invention DNA sequences encoding the mature region of the human BMP-9 protein may be joined to DNA sequences encoding the propeptide region of the murine BMP-9 protein. This murine/human hybrid DNA sequence is inserted into an appropriate expression vector and introduced into mammalian cells or other preferred eukaryotic or prokaryotic hosts by conventional genetic engineering techniques. The construction of this murine/human BMP-9 containing expression plasmid is described below.

A derivative of the human BMP-9 sequence (SEQ ID NO:8) comprising the nucleotide sequence from nucleotide #105 to #470 is specifically amplified. The following oligonucleotides are utilized as primers to allow the amplification of nucleotides #105 to #470 of the human BMP-9 sequence (SEQ ID NO:8) from clone pGEM-111 described above.

#3 ATCGGGCCCCTTTTAGCCAGGCGGAAAAGGAG

#4 AGCGAATCCCCGCAGGCAGATACTACCTG

This procedure generates the insertion of the nucleotide sequence ATCGGGCCCCT immediately preceding nucleotide #105 and the insertion of the nucleotide sequence GAATTCGCT immediately following nucleotide #470. The addition of these sequences results in the creation of an Apa I and EcoR I restriction endonuclease site at the respective ends of the specifically amplified DNA fragment. The resulting 374 bp Apa I/EcoR I fragment is subcloned into the plasmid vector pGEM-7Zf(+) (Promega catalog# p2251) which has been digested with Apa I and EcoR I. The resulting clone is designated pHBM9mex-1.

The following oligonucleotides are designed on the basis of murine BMP-9 sequences (SEQ ID NO:1) and are modified to facilitate the construction of the murine/human expression plasmid referred to above:

#5

GATTCCGTCGACCACCATGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC

#6 CCACAGCTGTGTATCCATCTAGACCAGGCCCCAGGGGACATGGTGGTTCGACG

These oligonucleotides contain complimentary sequences which upon addition to each other facilitate the annealing (base pairing) of the two individual sequences, resulting in the formation of a double stranded synthetic DNA linker (designated LINK-1) in a manner indicated below:

```

      1   5   10       20       30       40       50       60
      |   |   |       |       |       |       |       |
#5GATTCGTCGACCACTGTCCCCTGGGGCCTGGTCTAGATGGATACACAGCTGTGGGGCC
10      GCAGCTGGTGGTACAGGGGACCCCGGACCAGATCTACCTATGTGTCGACACC #6

```

This DNA linker (LINK-1) contains recognition sequences of restriction endonucleases needed to facilitate subsequent manipulations required to construct the murine/human expression plasmid, as well as sequences required for maximal expression of heterologous sequences in mammalian cell expression systems. More specifically (referring to the sequence numbering of oligonucleotide #5/LINK-1): nucleotides #1-#11 comprise recognition sequences for the restriction endonucleases BamH I and Sal I, nucleotides #11-#15 allow for maximal expression of heterologous sequences in mammalian cell expression systems, nucleotides #16-#31 correspond to nucleotides #610-#625 of the murine BMP-9 sequence (SEQ ID NO:1), nucleotides #32-#33 are inserted to facilitate efficient restriction digestion of two adjacent restriction endonuclease sites (EcoO109 I and Xba I), nucleotides #34-#60 correspond to nucleotides #1515-#1541 of the murine BMP-9 sequence (SEQ ID NO:1) except that nucleotide #58 of synthetic oligonucleotide #5 is a G rather than the A which appears at position #1539 of SEQ ID NO:1 (This nucleotide conversion results in the creation of an Apa I restriction

25

endonuclease recognition sequence, without altering the amino acid sequence it is intended to encode, to facilitate further manipulations of the murine/human hybrid expression plasmid. LINK-1 (the double stranded product of the annealing of oligonucleotides #5 and #6) is subcloned into the plasmid vector pGEM-7Zf(+) which has been digested with the restriction endonucleases Apa I and BamH I. This results in a plasmid in which the sequences normally present between the Apa I and BamH I sites of the pGEM-7Zf(+) plasmid polylinker are replaced with the sequences of LINK-1 described above. The resulting plasmid clone is designated pBMP-9link.

pBMP-9link is digested with the restriction endonucleases BamH I and Xba I resulting in the removal nucleotides #1-#34 of LINK-1 (refer to the numbering of oligo #5). Clone ML14a, which contains an insert comprising the sequence set forth in SEQ ID NO:1, is also digested with the restriction endonucleases BamH I and Xba I resulting in the removal of sequences comprising nucleotides #1-#1515 of SEQUENCE ID NO:1 (murine BMP-9). This BamH I/Xba I fragment of mouse BMP-9 is isolated from the remainder of the ML14a plasmid clone and subcloned into the BamH I/Xba I sites generated by the removal of the synthetic linker sequences described above. The resulting clone is designated p302.

The p302 clone is digested with the restriction endonuclease Eco0109 I resulting in the excision of nucleotides corresponding to nucleotides #621-#1515 of the murine BMP-9

SUBSTITUTE SHEET

sequence (SEQ ID NO:1) and nucleotides #35-#59 of LINK-1 (refer to numbering of oligonucleotide #5). It should be noted that the Apa I restriction site created in LINK-1 by the A to G conversion described above is a subset of the recognition sequence of EcoO109 I, therefore digestion of p302 with EcoO109 I cleaves at the Apa I site as well as the naturally occurring murine EcoO109 I (location #619-#625 of SEQ ID NO:1) resulting in the excision of a 920 bp EcoO109 I/EcoO109 I (Apa I) fragment comprising the sequences described above. This 920 EcoO109 I/EcoO109 I (Apa I) fragment is isolated from the remainder of the p302 plasmid clone and subcloned into clone pBMP-9link which has been similarly digested with EcoO109 I. It should be noted that the nucleotides GG (#32-#33 of oligonucleotide #5) originally designed to facilitate a more complete digestion of the two adjacent restriction sites EcoO109 I and Xba I of LINK-1, which is now a part of pBMP-9link (described above), results in the creation of Dcm methylation recognition sequence. The restriction nuclease EcoO109 I is sensitive to Dcm methylation and therefore cleavage of this sequence (nucleotides #25-#31 of oligonucleotide #5/LINK-1) by the restriction endonuclease EcoO109 I is prevented at this site. Therefore the plasmid clone pBMP-9link is cleaved at the Apa I site but not at the EcoO109 I site upon digestion with the restriction endonuclease EcoO109 I as described above, preventing the intended removal of the sequences between the EcoO109 I and Xba I site of LINK-1

(#32-#55 defined by the numbering of oligonucleotide #5). This results in the insertion of the 920 bp Eco0109 I/Apa I fragment at the Eco0109 I (Apa I) site of pBMP-9link. The resulting clone is designated p318.

5           Clone p318 is digested with the restriction endonucleases Sal I and Apa I, resulting in the excision of sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location), nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1), and nucleotides #35-#60 of LINK-1 (refer to oligo #5 for  
10 location). The resulting 972 bp Sal I/Apa I fragment described above is isolated from the remainder of the p318 plasmid clone and will be utilized in subsequent manipulations.

          The clone phBMP9mex-1 (described above), which contains DNA sequences which encode the entire mature region and  
15 portions of the propeptide of the human BMP-9 protein, is digested with the restriction endonucleases Apa I and EcoR I. This results in the excision of a 374 bp fragment comprising nucleotides #105-#470 of the human BMP-9 sequence (SEQ ID NO:8) and the additional nucleotides of oligonucleotide primers #3  
20 and #4 which contain the recognition sequences for the restriction endonucleases Apa I and EcoR I. This 374 bp Apa I/EcoR I fragment is combined with the 972 bp Sal I/Apa I fragment from p318 (isolation described above) and ligated to the mammalian cell expression plasmid pED6 (a derivative of  
25 pEMC2 $\beta$ 1) which has been digested with Sal I and EcoR I. The resulting clone is designated p324.

The clone ML14a (murine BMP-9) is digested with EcoO109 I and Xba I to generate a fragment comprising nucleotides #621-#1515 of SEQ ID NO:1.

The following oligonucleotides are synthesized on an automated DNA synthesizer and combined such that their complimentary sequences can base pair (anneal) with each other to generate a double stranded synthetic DNA linker designated LINK-2:

#7 TCGACCACCATGTCCCCTGG

10 #8 GCCCCAGGGGACATGGTGG

This double stranded synthetic DNA linker (LINK-2) anneals in such a way that it generates single stranded ends which are compatible to DNA fragments digested with Sal I (one end) or EcoO109 I (the other end) as indicated below:

15 #7 TCGACCACCATGTCCCCTGG  
GGTGGTACAGGGGACCCCG #8

This LINK-2 synthetic DNA linker is ligated to the 895 bp EcoO109 I/Xba I fragment comprising nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) described above. This results in a 915 bp Sal I/Xba I fragment.

The clone p324 is digested with Sal I/Xba I to remove sequences comprising nucleotides #6-#56 of LINK-1 (refer to oligo #5 for location) and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1). The sequences comprising nucleotides #35-#60 of LINK-1 (refer to oligo #5 for location) and the sequences comprising the 374 bp Apa I/EcoR I fragment (human BMP-9 sequences) derived from phBMP9mex-1 remain attached to

the pED6 backbone. The 915 bp Sal I/Xba I fragment comprising LINK-2 sequences and nucleotides #621-#1515 of murine BMP-9 (SEQ ID NO:1) is ligated into the p324 clone from which the Sal I to Xba I sequences described above have been removed.

5       The resulting plasmid is designated BMP9fusion and comprises LINK-2, nucleotides #621-#1551 of murine BMP-9 (SEQ ID NO:1), nucleotides #35-#59 of LINK-1 (refer to the numbering of oligonucleotide #5), and the 374 bp Apa I/EcoR I fragment (human BMP-9) derived from clone pBMP9mex-1 (described above)  
10       inserted between the Sal I and EcoR I sites of the mammalian cell expression vector pED6.

BMP9 fusion is transfected into CHO cells using standard techniques known to those having ordinary skill in the art to create stable cell lines capable of expressing human BMP-9  
15       protein. The cell lines are cultured under suitable culture conditions and the BMP-9 protein is isolated and purified from the culture medium.

#### EXAMPLE V

##### 20       Biological Activity of Expressed BMP-9

To measure the biological activity of the expressed BMP-9 proteins obtained in Example IV above, the proteins are recovered from the cell culture and purified by isolating the BMP-9 proteins from other proteinaceous materials with which  
25       they are co-produced as well as from other contaminants. The purified protein may be assayed in accordance with the rat bone

formation assay described in Example III.

Purification is carried out using standard techniques known to those skilled in the art. It is contemplated, as with other BMP proteins, that purification may include the use of Heparin sepharose.

Protein analysis is conducted using standard techniques such as SDS-PAGE acrylamide [U.K. Laemmli, Nature 227:680 (1970)] stained with silver [R.R. Oakley, et al. Anal. Biochem. 105:361 (1980)] and by immunoblot [H. Towbin, et al. Proc. Natl. Acad. Sci. USA 76:4350 (1979)]

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.



## (1) GENERAL INFORMATION:

- (i) APPLICANT: Wozney, John M.  
Celeste, Anthony
- (ii) TITLE OF INVENTION: BMP-9 COMPOSITIONS
- (iii) NUMBER OF SEQUENCES: 9
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Genetics Institute, Inc.
  - (B) STREET: Legal Affairs - 87 CambridgePark Drive
  - (C) CITY: Cambridge
  - (D) STATE: MA
  - (E) COUNTRY: US
  - (F) ZIP: 02140
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Kapinos, Ellen J.
  - (B) REGISTRATION NUMBER: 32,245
  - (C) REFERENCE/DOCKET NUMBER: GI 5186A
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (617) 876-1170
  - (B) TELEFAX: (617) 876-5851

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2447 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Mus musculus
  - (B) STRAIN: C57B46xCBA
  - (F) TISSUE TYPE: liver

SUBSTITUTE SHEET

(vii) IMMEDIATE SOURCE:  
 (A) LIBRARY: Mouse liver cDNA  
 (B) CLONE: ML14A

(viii) POSITION IN GENOME:  
 (C) UNITS: bp

(ix) FEATURE:  
 (A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 1564..1893

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 610..1896

(ix) FEATURE:  
 (A) NAME/KEY: mRNA  
 (B) LOCATION: 1..2447

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CATTAATAAA TATTAAGTAT TGGAATTAGT GAAATTGGAG TTCCTTGTGG AAGGAAGTGG	60
GCAAGTGAGC TTTTTAGTTT GTGTCGGAAG CCTGTAATTA CGGCTCCAGC TCATAGTGGA	120
ATGGCTATAC TTAGATTTAT GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG	180
CCTAGGAGAT TTGTTGATCC AATAAATATG ATTAGGGAAA CAATTATTAG GGTTCATGTT	240
CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTTTGATAA TAAAGTTTAAC TAGTCAGTGT	300
TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGAATAG GATTGAAGGA	360
AATATAATGA TGGCTACAAC GATTGGGAAT CCTATTATTG TTGGGGTAAT GAATGAGGCA	420
AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA	480
TTGGTGAGTA GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA	540
CCTGATGTTA GAAGGAGGGC TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG	600
GCCACCCGG ATG TCC CCT GGG GCC TTC CGG GTG GCC CTG CTC CCG CTG	648
Met Ser Pro Gly Ala Phe Arg Val Ala Leu Leu Pro Leu	
-318 -315 -310	
TTC CTG CTG GTC TGT GTC ACA CAG CAG AAG CCG CTG CAG AAC TGG GAA	696
Phe Leu Leu Val Cys Val Thr Gln Gln Lys Pro Leu Gln Asn Trp Glu	
-305 -300 -295 -290	
CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC CTG GGA TTG TCT GGA	744
Gln Ala Ser Pro Gly Glu Asn Ala His Ser Ser Leu Gly Leu Ser Gly	
-285 -280 -275	
GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG GAG AAC ATG	792
Ala Gly Glu Glu Gly Val Phe Asp Leu Gln Met Phe Leu Glu Asn Met	
-270 -265 -260	
AAG GTG GAT TTC CTA CGC AGC CTT AAC CTC AGC GGC ATT CCC TCC CAG	840
Lys Val Asp Phe Leu Arg Ser Leu Asn Leu Ser Gly Ile Pro Ser Gln	

SUBSTITUTE SHEET

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 151 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

*  Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg
-41 -40                               -35                -30

Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala
-25                               -20                -15                -10

Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His
                               -5                1                5

Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp
                               10                15                20

Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly
                25                30                35

Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala
40                45                50                55

Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys
                60                65                70

Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys
                75                80                85

Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser
                90                95                100

Val Ala Glu Cys Gly Cys Arg
105                110

```

SUBSTITUTE SHEET

## (ix) FEATURE:

(A) NAME/KEY: exon  
(B) LOCATION: 1..470

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 1..456

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 124..453

## (ix) FEATURE:

(A) NAME/KEY: mRNA  
(B) LOCATION: 1..470

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGA ACA AGA GAG TGC TCA AGA AGC TGT CCA AGG ACG GCT CCA CAG AGG	48
* Thr Arg Glu Cys Ser Arg Ser Cys Pro Arg Thr Ala Pro Gln Arg	
-41 -40 -35 -30	
CAG GTG AGA GCA GTC ACG AGG AGG ACA CGG ATG GCG CAC GTG GCT GCG	96
Gln Val Arg Ala Val Thr Arg Arg Thr Arg Met Ala His Val Ala Ala	
-25 -20 -15 -10	
GGG TCG ACT TTA GCC AGG CGG AAA AGG AGC GCC GGG GCT GGC AGC CAC	144
Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala Gly Ser His	
-5 1 5	
TGT CAA AAG ACC TCC CTG CGG GTA AAC TTC GAG GAC ATC GGC TGG GAC	192
Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile Gly Trp Asp	
10 15 20	
AGC TGG ATC ATT GCA CCC AAG GAG TAT GAA GCC TAC GAG TGT AAG GGC	240
Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu Cys Lys Gly	
25 30 35	
GGC TGC TTC TTC CCC TTG GCT GAC GAT GTG ACG CCG ACG AAA CAC GCT	288
Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala	
40 45 50 55	
ATC GTG CAG ACC CTG GTG CAT CTC AAG TTC CCC ACA AAG GTG GGC AAG	336
Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys Val Gly Lys	
60 65 70	
GCC TGC TGT GTG CCC ACC AAA CTG AGC CCC ATC TCC GTC CTC TAC AAG	384
Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val Leu Tyr Lys	
75 80 85	
GAT GAC ATG GGG GTG CCC ACC CTC AAG TAC CAT TAC GAG GGC ATG AGC	432
Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu Gly Met Ser	
90 95 100	
GTG GCA GAG TGT GGG TGC AGG TAGTATCTGC CTGCGGG	470
Val Ala Glu Cys Gly Cys Arg	
105 110	

CATGGGCAGC TCGAG

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGCAGGCCGA GCCTGAATTC CTCGAGCCAT CATG

34

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 68 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGAGGTAAA AAACGTCTAG GCCCCCGAA CCACGGGGAC GTGGTTTTCC TTTGAAAAAC  
ACGATTGC

60

68

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 470 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (H) CELL LINE: W138 (genomic DNA)

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: human genomic library
- (B) CLONE: lambda 111-1

(viii) POSITION IN GENOME:

- (C) UNITS: bp

SUBSTITUTE SHEET

Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro  
 -115 -110 -105  
 Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn  
 -100 -95 -90 -85  
 Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp  
 -80 -75 -70  
 Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His  
 -65 -60 -55  
 Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg  
 -50 -45 -40  
 Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu  
 -35 -30 -25  
 Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg  
 -20 -15 -10 -5  
 Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys  
 1 5 10  
 Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val  
 15 20 25  
 Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr  
 30 35 40  
 Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 45 50 55 60  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile  
 65 70 75  
 Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu  
 80 85 90  
 Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met  
 95 100 105  
 Val Val Glu Gly Cys Gly Cys Arg  
 110 115

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

SUBSTITUTE SHEET

TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC 1666  
 Cys Gly Cys Arg  
 115

CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC 1726

ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA 1786

ATCCCTAAAC ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAAATGTT TTGACCATAT 1846

TGATCATATA TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG 1906

AGTCATTATT TTAATAAAAA AAAAAAACT CTAGAGTCGA CGGAATTC 1954

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 408 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val  
 -292 -290 -285 -280

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys  
 -275 -270 -265

Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly  
 -260 -255 -250 -245

Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met  
 -240 -235 -230

Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro  
 -225 -220 -215

Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu  
 -210 -205 -200

Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser  
 -195 -190 -185

Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn  
 -180 -175 -170 -165

Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu  
 -160 -155 -150

Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu  
 -145 -140 -135

Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His  
 -130 -125 -120

SUBSTITUTE SHEET

GTG	GAC	CAG	GGC	CCT	GAT	TGG	GAA	AGG	GGC	TTC	CAC	CGT	ATA	AAC	ATT	942
Val	Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His	Arg	Ile	Asn	Ile	
			-125					-120					-115			
TAT	GAG	GTT	ATG	AAG	CCC	CCA	GCA	GAA	GTG	GTG	CCT	GGG	CAC	CTC	ATC	990
Tyr	Glu	Val	Met	Lys	Pro	Pro	Ala	Glu	Val	Val	Pro	Gly	His	Leu	Ile	
		-110					-105					-100				
ACA	CGA	CTA	CTG	GAC	ACG	AGA	CTG	GTC	CAC	CAC	AAT	GTG	ACA	CGG	TGG	1038
Thr	Arg	Leu	Leu	Asp	Thr	Arg	Leu	Val	His	His	Asn	Val	Thr	Arg	Trp	
	-95					-90					-85					
GAA	ACT	TTT	GAT	GTG	AGC	CCT	GCG	GTC	CTT	CGC	TGG	ACC	CGG	GAG	AAG	1086
Glu	Thr	Phe	Asp	Val	Ser	Pro	Ala	Val	Leu	Arg	Trp	Thr	Arg	Glu	Lys	
-80					-75					-70					-65	
CAG	CCA	AAC	TAT	GGG	CTA	GCC	ATT	GAG	GTG	ACT	CAC	CTC	CAT	CAG	ACT	1134
Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu	Val	Thr	His	Leu	His	Gln	Thr	
				-60				-55						-50		
CGG	ACC	CAC	CAG	GGC	CAG	CAT	GTC	AGG	ATT	AGC	CGA	TCG	TTA	CCT	CAA	1182
Arg	Thr	His	Gln	Gly	Gln	His	Val	Arg	Ile	Ser	Arg	Ser	Leu	Pro	Gln	
			-45				-40						-35			
GGG	AGT	GGG	AAT	TGG	GCC	CAG	CTC	CGG	CCC	CTC	CTG	GTC	ACC	TTT	GGC	1230
Gly	Ser	Gly	Asn	Trp	Ala	Gln	Leu	Arg	Pro	Leu	Leu	Val	Thr	Phe	Gly	
		-30					-25					-20				
CAT	GAT	GGC	CGG	GGC	CAT	GCC	TTG	ACC	CGA	CGC	CGG	AGG	GCC	AAG	CGT	1278
His	Asp	Gly	Arg	Gly	His	Ala	Leu	Thr	Arg	Arg	Arg	Arg	Ala	Lys	Arg	
	-15					-10					-5					
AGC	CCT	AAG	CAT	CAC	TCA	CAG	CGG	GCC	AGG	AAG	AAG	AAT	AAG	AAC	TGC	1326
Ser	Pro	Lys	His	His	Ser	Gln	Arg	Ala	Arg	Lys	Lys	Asn	Lys	Asn	Cys	
1				5				10						15		
CGG	CGC	CAC	TCG	CTC	TAT	GTG	GAC	TTC	AGC	GAT	GTG	GGC	TGG	AAT	GAC	1374
Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	Trp	Asn	Asp	
			20					25					30			
TGG	ATT	GTG	GCC	CCA	CCA	GGC	TAC	CAG	GCC	TTC	TAC	TGC	CAT	GGG	GAC	1422
Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	Gln	Ala	Phe	Tyr	Cys	His	Gly	Asp	
		35				40						45				
TGC	CCC	TTT	CCA	CTG	GCT	GAC	CAC	CTC	AAC	TCA	ACC	AAC	CAT	GCC	ATT	1470
Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser	Thr	Asn	His	Ala	Ile	
	50					55					60					
GTG	CAG	ACC	CTG	GTC	AAT	TCT	GTC	AAT	TCC	AGT	ATC	CCC	AAA	GCC	TGT	1518
Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn	Ser	Ser	Ile	Pro	Lys	Ala	Cys	
65					70				75						80	
TGT	GTG	CCC	ACT	GAA	CTG	AGT	GCC	ATC	TCC	ATG	CTG	TAC	CTG	GAT	GAG	1566
Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	Tyr	Leu	Asp	Glu	
				85				90						95		
TAT	GAT	AAG	GTG	GTA	CTG	AAA	AAT	TAT	CAG	GAG	ATG	GTA	GTA	GAG	GGA	1614
Tyr	Asp	Lys	Val	Val	Leu	Lys	Asn	Tyr	Gln	Glu	Met	Val	Val	Glu	Gly	
			100					105					110			

SUBSTITUTE SHEET



(B) LOCATION: 9..1934

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA	60
GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG	120
AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC	180
ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG	240
CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC	300
GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA	360
TCATGGACTG TTATTATATG CCTTGTTTTT TGTCAAGACA CC ATG ATT CCT GGT	414
	Met Ile Pro Gly
	-292 -290
AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC	462
Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly	
	-285 -280 -275
GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC	510
Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala	
	-270 -265 -260
GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG	558
Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu	
	-255 -250 -245
CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC	606
Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg	
	-240 -235 -230 -225
CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG	654
Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg	
	-220 -215 -210
GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAG GAA GAG CAG ATC CAC	702
Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Glu Gln Ile His	
	-205 -200 -195
AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC	750
Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr	
	-190 -185 -180
GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC	798
Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr	
	-175 -170 -165
AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT	846
Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro	
	-160 -155 -150 -145
GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG	894
Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln	
	-140 -135 -130

SUBSTITUTE SHEET

-10

-5

1

Gly	Ala	Ser	Ser	His	Cys	Gln	Lys	Thr	Ser	Leu	Arg	Val	Asn	Phe	Glu
		5					10					15			
Asp	Ile	Gly	Trp	Asp	Ser	Trp	Ile	Ile	Ala	Pro	Lys	Glu	Tyr	Asp	Ala
	20					25					30				
Tyr	Glu	Cys	Lys	Gly	Gly	Cys	Phe	Phe	Pro	Leu	Ala	Asp	Asp	Val	Thr
	35				40					45					50
Pro	Thr	Lys	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Glu	Phe	Pro
				55					60					65	
Thr	Lys	Val	Gly	Lys	Ala	Cys	Cys	Val	Pro	Thr	Lys	Leu	Ser	Pro	Ile
			70					75					80		
Ser	Ile	Leu	Tyr	Lys	Asp	Asp	Met	Gly	Val	Pro	Thr	Leu	Lys	Tyr	His
		85					90					95			
Tyr	Glu	Gly	Met	Ser	Val	Ala	Glu	Cys	Gly	Cys	Arg				
	100					105					110				

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1954 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (G) CELL TYPE: Osteosarcoma Cell Line
- (H) CELL LINE: U-2OS

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: U2OS cDNA in Lambda gt10
- (B) CLONE: Lambda U2OS-3

## (viii) POSITION IN GENOME:

- (C) UNITS: bp

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 403..1629

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 1279..1626

## (ix) FEATURE:

- (A) NAME/KEY: mRNA

SUBSTITUTE SHEET

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Pro Gly Ala Phe Arg Val Ala Leu Leu Pro Leu Phe Leu Leu  
 -318                      -315                      -310                      -305  
 Val Cys Val Thr Gln Gln Lys Pro Leu Gln Asn Trp Glu Gln Ala Ser  
                     -300                      -295                      -290  
 Pro Gly Glu Asn Ala His Ser Ser Leu Gly Leu Ser Gly Ala Gly Glu  
                     -285                      -280                      -275  
 Glu Gly Val Phe Asp Leu Gln Met Phe Leu Glu Asn Met Lys Val Asp  
 -270                      -265                      -260                      -255  
 Phe Leu Arg Ser Leu Asn Leu Ser Gly Ile Pro Ser Gln Asp Lys Thr  
                     -250                      -245                      -240  
 Arg Ala Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr  
                     -235                      -230                      -225  
 Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser  
                     -220                      -215                      -210  
 Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe Pro Phe Gln  
                     -205                      -200                      -195  
 Lys His Ile Leu Ile Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile  
 -190                      -185                      -180                      -175  
 Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn Asp Val Asp  
                     -170                      -165                      -160  
 Ser Thr His Gly Leu Glu Gly Ser Met Val Val Tyr Asp Val Leu Glu  
                     -155                      -150                      -145  
 Asp Ser Glu Thr Trp Asp Gln Ala Thr Gly Thr Lys Thr Phe Leu Val  
                     -140                      -135                      -130  
 Ser Gln Asp Ile Arg Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser  
                     -125                      -120                      -115  
 Ala Val Lys Arg Trp Val Arg Ala Asp Ser Thr Thr Asn Lys Asn Lys  
 -110                      -105                      -100                      -95  
 Leu Glu Val Thr Val Gln Ser His Arg Glu Ser Cys Asp Thr Leu Asp  
                     -90                      -85                      -80  
 Ile Ser Val Pro Pro Gly Ser Lys Asn Leu Pro Phe Phe Val Val Phe  
                     -75                      -70                      -65  
 Ser Asn Asp Arg Ser Asn Gly Thr Lys Glu Thr Arg Leu Glu Leu Lys  
                     -60                      -55                      -50  
 Glu Met Ile Gly His Glu Gln Glu Thr Met Leu Val Lys Thr Ala Lys  
                     -45                      -40                      -35  
 Asn Ala Tyr Gln Val Ala Gly Glu Ser Gln Glu Glu Glu Gly Leu Asp  
 -30                      -25                      -20                      -15  
 Gly Tyr Thr Ala Val Gly Pro Leu Leu Ala Arg Arg Lys Arg Ser Thr

SUBSTITUTE SHEET

-15	-10	-5	
AGG AGC ACC GGA GCC AGC AGC CAC TGC CAG AAG ACT TCT CTC AGG GTG Arg Ser Thr Gly Ala Ser Ser His Cys Gln Lys Thr Ser Leu Arg Val 1 5 10 15			1608
AAC TTT GAG GAC ATC GGC TGG GAC AGC TGG ATC ATT GCA CCC AAG GAA Asn Phe Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu 20 25 30			1656
TAT GAC GCC TAT GAG TGT AAA GGG GGT TGC TTC TTC CCA TTG GCT GAT Tyr Asp Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp 35 40 45			1704
GAC GTG ACA CCC ACC AAA CAT GCC ATC GTG CAG ACC CTG GTG CAT CTC Asp Val Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu 50 55 60			1752
GAG TTC CCC ACA AAG GTG GGC AAA GCC TGC TGC GTT CCC ACC AAA CTG Glu Phe Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu 65 70 75			1800
AGT CCC ATC TCC ATC CTC TAC AAG GAT GAC ATG GGG GTG CCA ACC CTC Ser Pro Ile Ser Ile Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu 80 85 90 95			1848
AAG TAC CAC TAT GAG GGG ATG AGT GTG GCT GAG TGT GGG TGT AGG TAGTCCCTGC Lys Tyr His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg 100 105 110			1903
AGCCACCCAG GGTGGGGATA CAGGACATGG AAGAGGTTCT GGTACGGTCC TGCATCCTCC			1963
TGCGCATGGT ATGCCTAAGT TGATCAGAAA CCATCCTTGA GAAGAAAAGG AGTTAGTTGC			2023
CCTTCTTGTG TCTGGTGGGT CCCTCTGCTG AAGTGACAAT GACTGGGGTA TGCGGGCCTG			2083
TGGGCAGAGC AGGAGACCCT GGAAGGGTTA GTGGGTAGAA AGATGTCAAA AAGGAAGCTG			2143
TGGGTAGATG ACCTGCACTC CAGTGATTAG AAGTCCAGCC TTACCTGTGA GAGAGCTCCT			2203
GGCATCTAAG AGAACTCTGC TTCCTCATCA TCCCCACCGA CTTGTTCTTC CTTGGGAGTG			2263
TGTCCTCAGG GAGAACAGCA TTGCTGTTCC TGTGCCTCAA GCTCCCAGCT GACTCTCCTG			2323
TGGCTCATAG GACTGAATGG GGTGAGGAAG AGCCTGATGC CCTCTGGCAA TCAGAGCCCCG			2383
AAGGACTTCA AAACATCTGG ACAACTCTCA TTGACTGATG CTCCAACATA ATTTTAAAAA			2443
AGAG			2447

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 428 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

SUBSTITUTE SHEET

-255	-250	-245	
GAC AAA ACC AGA GCG GAG CCA CCC CAG TAC ATG ATC GAC TTG TAC AAC			888
Asp Lys Thr Arg Ala Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn			
-240	-235	-230	
AGA TAC ACA ACG GAC AAA TCG TCT ACG CCT GCC TCC AAC ATC GTG CGG			936
Arg Tyr Thr Thr Asp Lys Ser Ser Thr Pro Ala Ser Asn Ile Val Arg			
-225	-220	-215	-210
AGC TTC AGC GTG GAA GAT GCT ATA TCG ACA GCT GCC ACG GAG GAC TTC			984
Ser Phe Ser Val Glu Asp Ala Ile Ser Thr Ala Ala Thr Glu Asp Phe			
-205	-200	-195	
CCC TTT CAG AAG CAC ATC CTG ATC TTC AAC ATC TCC ATC CCG AGG CAC			1032
Pro Phe Gln Lys His Ile Leu Ile Phe Asn Ile Ser Ile Pro Arg His			
-190	-185	-180	
GAG CAG ATC ACC AGG GCT GAG CTC CGA CTC TAT GTC TCC TGC CAA AAT			1080
Glu Gln Ile Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn			
-175	-170	-165	
GAT GTG GAC TCC ACT CAT GGG CTG GAA GGA AGC ATG GTC GTT TAT GAT			1128
Asp Val Asp Ser Thr His Gly Leu Glu Gly Ser Met Val Val Tyr Asp			
-160	-155	-150	
GTT CTG GAG GAC AGT GAG ACT TGG GAC CAG GCC ACG GGG ACC AAG ACC			1176
Val Leu Glu Asp Ser Glu Thr Trp Asp Gln Ala Thr Gly Thr Lys Thr			
-145	-140	-135	-130
TTC TTG GTA TCC CAG GAC ATT CGG GAC GAA GGA TGG GAG ACT TTA GAA			1224
Phe Leu Val Ser Gln Asp Ile Arg Asp Glu Gly Trp Glu Thr Leu Glu			
-125	-120	-115	
GTA TCG AGT GCC GTG AAG CGG TGG GTC AGG GCA GAC TCC ACA ACA AAC			1272
Val Ser Ser Ala Val Lys Arg Trp Val Arg Ala Asp Ser Thr Thr Asn			
-110	-105	-100	
AAA AAT AAG CTC GAG GTG ACA GTG CAG AGC CAC AGG GAG AGC TGT GAC			1320
Lys Asn Lys Leu Glu Val Thr Val Gln Ser His Arg Glu Ser Cys Asp			
-95	-90	-85	
ACA CTG GAC ATC AGT GTC CCT CCA GGT TCC AAA AAC CTG CCC TTC TTT			1368
Thr Leu Asp Ile Ser Val Pro Pro Gly Ser Lys Asn Leu Pro Phe Phe			
-80	-75	-70	
GTT GTC TTC TCC AAT GAC CGC AGC AAT GGG ACC AAG GAG ACC AGA CTG			1416
Val Val Phe Ser Asn Asp Arg Ser Asn Gly Thr Lys Glu Thr Arg Leu			
-65	-60	-55	-50
GAG CTG AAG GAG ATG ATC GGC CAT GAG CAG GAG ACC ATG CTT GTG AAG			1464
Glu Leu Lys Glu Met Ile Gly His Glu Gln Glu Thr Met Leu Val Lys			
-45	-40	-35	
ACA GCC AAA AAT GCT TAC CAG GTG GCA GGT GAG AGC CAA GAG GAG GAG			1512
Thr Ala Lys Asn Ala Tyr Gln Val Ala Gly Glu Ser Gln Glu Glu Glu			
-30	-25	-20	
GGT CTA GAT GGA TAC ACA GCT GTG GGA CCA CTT TTA GCT AGA AGG AAG			1560
Gly Leu Asp Gly Tyr Thr Ala Val Gly Pro Leu Leu Ala Arg Arg Lys			

SUBSTITUTE SHEET

What is claimed is:

1. A BMP-9 polypeptide comprising the amino acid sequence from amino acid #8 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).
2. A BMP-9 polypeptide comprising the amino acid sequence from amino acid #1 - 110 as set forth in FIG. 3 (SEQ ID NO: 9).
3. A BMP-9 polypeptide of claim 1 wherein said polypeptide is a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #8 - 110 of FIG. 3 (SEQ ID NO: 9).
4. A BMP-9 polypeptide of claim 2 wherein said polypeptide is a dimer wherein each subunit comprises at least the amino acid sequence from amino acid #1-110 of FIG. 3. (SEQ ID NO: 9).
5. A purified BMP-9 protein produced by the steps of
  - (a) culturing a cell transformed with a cDNA comprising the nucleotide sequence from nucleotide #124 to #453 as shown in FIG. 3 (SEQ ID NO: 8); and
  - (b) recovering and purifying from said culture medium a protein comprising the amino acid sequence from amino acid #1 to amino acid #110 as shown in FIG. 3 (SEQ ID NO: 9).
6. A purified BMP-9 protein produced by the steps of
  - (a) culturing a cell transformed with a cDNA comprising

the nucleotide sequence from nucleotide #124 to #453 as shown in FIG. 3 (SEQ ID NO: 8); and

(b) recovering from said culture medium a protein comprising an amino acid sequence from amino acid #8 to amino acid #110 as shown in Figure 3 (SEQ ID NO: 9).

7. A BMP-9 protein characterized by the ability to induce the formation of cartilage and/or bone.

8. A DNA sequence encoding a BMP-9 protein.

9. The DNA sequence of claim 8 wherein said DNA comprises

(a) nucleotide 124 to 453 (SEQ ID NO: 8); and

(b) sequences which hybridize thereto under stringent hybridization conditions and exhibit the ability to form cartilage and/or bone.

10. The DNA sequence of claim 8 wherein said DNA comprises

(a) nucleotide 145 to 453 (SEQ ID NO: 8); and

(b) sequences which hybridize thereto under stringent hybridization conditions and exhibit the ability to form cartilage and/or bone.

11. A host cell transformed with a DNA sequence encoding BMP-8.

12. A method for producing a purified BMP-9 protein said method comprising the steps of

(a) culturing a cell transformed with a cDNA comprising the nucleotide sequence encoding a BMP-9 protein; and

(b) recovering and purifying said BMP-9 protein from the culture medium.

13. A pharmaceutical composition comprising an effective amount of a BMP-9 protein in admixture with a pharmaceutically acceptable vehicle.

14. A composition of claim 13 further comprising a matrix for supporting said composition and providing a surface for bone and/or cartilage growth.

15. The composition of claim 14 wherein said matrix comprises a material selected from the group consisting of hydroxyapatite, collagen, polylactic acid and tricalcium phosphate.

16. A method for inducing bone and/or cartilage formation in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 13.

17. A pharmaceutical composition for wound healing and tissue repair said composition comprising an effective amount of the



protein of a BMP-9 protein in a pharmaceutically acceptable vehicle.

18. A method for treating wounds and/or tissue repair in a patient in need of same comprising administering to said patient an effective amount of the composition of claim 17.

1/8

Figure 1A

10 20 30 40 50 60 70  
 CATTAATAAA TATTAAGTAT TGAATTAGT GAAATTGGAG TTCCTTGTGG AAGGAAGTGG GCAAGTGAGC  
 80 90 100 110 120 130 140  
 TTTTATGTTT GTGTCGGAAG CCTGTAATTA CGGCTCCAGC TCATAGTGGA ATGGCTATAC TTAGATTTAT  
 150 160 170 180 190 200 210  
 GGATAGTTGG GTAGTAGGTG TAAATGTATG TGGTAAAAGG CCTAGGAGAT TTGTTGATCC AATAAATATG  
 220 230 240 250 260 270 280  
 ATTAGGGAAA CAATTATTAG GGTTCATGTT CGTCCTTTTG GTGTGTGGAT TAGCATTATT TGTGTTGATAA  
 290 300 310 320 330 340 350  
 TAAGTTTAAAC TAGTCAGTGT TGGAAAGAAT GGAGACGGTT GTTGATTAGG CGTTTTGAGG ATGGGAATAG  
 360 370 380 390 400 410 420  
 GATTGAAGGA AATATAATGA TGGCTACAAC GATTGGGAAT CCTATTATG TTGGGGTAAT GAATGAGGCA  
 430 440 450 460 470 480 490  
 AATAGATTTT CGTTCATTTT AATTCTCAAG GGGTTTTTAC TTTTATGTTT GTTAGTGATA TTGGTGAGTA  
 500 510 520 530 540 550 560  
 GGCCAAGGGT TAATAGTGTA ATTGAATTAT AGTGAAATCA TATTACTAGA CCTGATGTGA GAAGGAGGGC  
 570 580 590 600 609 618  
 TGAAAAGGCT CCTTCCCTCC CAGGACAAAA CCGGAGCAGG GCCACCCGG <sup>></sup>ATG TCC CCT GGG  
 M S P G  
 627 636 645 654 663 672  
 GCC TTC CGG GTG GCC CTG CTC CCG CTG TTC CTG CTG GTC TGT GTC ACA CAG CAG  
 A F R V A L L P L F L L V C V T Q Q  
 681 690 699 708 717 726  
 AAG CCG CTG CAG AAC TGG GAA CAA GCA TCC CCT GGG GAA AAT GCC CAC AGC TCC  
 K P L Q N W E Q A S P G E N A H S S  
 735 744 753 762 771 780  
 CTG GGA TTG TCT GGA GCT GGA GAG GAG GGT GTC TTT GAC CTG CAG ATG TTC CTG  
 L G L S G A G E E G V F D L Q M F L  
 789 798 807 816 825 834  
 GAG AAC ATG AAG GTG GAT TTC CTA CGC AGC CTT AAC CTC AGC GGC ATT CCC TCC  
 E N M K V D F L R S L N L S G I P S

2/8

Figure 1B

843				852				861				870				879				888			
CAG Q	GAC D	AAA K	ACC T	AGA R	GCG A	GAG E	CCA P	CCC P	CAG Q	TAC Y	ATG M	ATC I	GAC D	TTG L	TAC Y	AAC N	AGA R						
897				906				915				924				933				942			
TAC Y	ACA T	ACG T	GAC D	AAA K	TCG S	TCT S	ACG T	CCT P	GCC A	TCC S	AAC N	ATC I	GTG V	CGG R	AGC S	TTC F	AGC S						
951				960				969				978				987				996			
GTG V	GAA E	GAT D	GCT A	ATA I	TCG S	ACA T	GCT A	GCC A	ACG T	GAG E	GAC D	TTC F	CCC P	TTT F	CAG Q	AAG K	CAC H						
1005				1014				1023				1032				1041				1050			
ATC I	CTG L	ATC I	TTC F	AAC N	ATC I	TCC S	ATC I	CCG P	AGG R	CAC H	GAG E	CAG Q	ATC I	ACC T	AGG R	GCT A	GAG E						
1059				1068				1077				1086				1095				1104			
CTC L	CGA R	CTC L	TAT Y	GTC V	TCC S	TGC C	CAA Q	AAT N	GAT D	GTG V	GAC D	TCC S	ACT T	CAT H	GGG G	CTG L	GAA E						
1113				1122				1131				1140				1149				1158			
GGA G	AGC S	ATG M	GTC V	GTT V	TAT Y	GAT D	GTT V	CTG L	GAG E	GAC D	AGT S	GAG E	ACT T	TGG W	GAC D	CAG Q	GCC A						
1167				1176				1185				1194				1203				1212			
ACG T	GGG G	ACC T	AAG K	ACC T	TTC F	TTG L	GTA V	TCC S	CAG Q	GAC D	ATT I	CGG R	GAC D	GAA E	GGA G	TGG W	GAG E						
1221				1230				1239				1248				1257				1266			
ACT T	TTA L	GAA E	GTA V	TCG S	AGT S	GCC A	GTG V	AAG K	CGG R	TGG W	GTC V	AGG R	GCA A	GAC D	TCC S	ACA T	ACA T						
1275				1284				1293				1302				1311				1320			
AAC N	AAA K	AAT N	AAG K	CTC L	GAG E	GTG V	ACA T	GTG V	CAG Q	AGC S	CAC H	AGG R	GAG E	AGC S	TGT C	GAC D	ACA T						
1329				1338				1347				1356				1365				1374			
CTG L	GAC D	ATC I	AGT S	GTC V	CCT P	CCA P	GGT G	TCC S	AAA K	AAC N	CTG L	CCC P	TTC F	TTT F	GTT V	GTC V	TTC F						

SUBSTITUTE SHEET

3/8

Figure 1C

1383	1392	1401	1410	1419	1428
TCC AAT GAC CGC AGC AAT GGG ACC AAG GAG ACC AGA CTG GAG CTG AAG GAG ATG					
S N D R S N G T K E T R L E L K E M					
1437	1446	1455	1464	1473	1482
ATC GGC CAT GAG CAG GAG ACC ATG CTT GTG AAG ACA GCC AAA AAT GCT TAC CAG					
I G H E Q E T M L V K T A K N A Y Q					
1491	1500	1509	1518	1527	1536
GTG GCA GGT GAG AGC CAA GAG GAG GAG GGT CTA GAT GGA TAC ACA GCT GTG GGA					
V A G E S Q E E E G L D G Y T A V G					
1545	1554	1563	1572	1581	1590
CCA CTT TTA GCT AGA AGG AAG AGG AGC ACC GGA GCC AGC AGC CAC TGC CAG AAG					
P L L A R R K R S T G A S S H C Q K					
1599	1608	1617	1626	1635	1644
ACT TCT CTC AGG GTG AAC TTT GAG GAC ATC GGC TGG GAC AGC TGG ATC ATT GCA					
T S L R V N F E D I G W D S W I I A					
1653	1662	1671	1680	1689	1698
CCC AAG GAA TAT GAC GCC TAT GAG TGT AAA GGG GGT TGC TTC TTC CCA TTG GCT					
P K E Y D A Y E C K G G C F F P L A					
1707	1716	1725	1734	1743	1752
GAT GAC GTG ACA CCC ACC AAA CAT GCC ATC GTG CAG ACC CTG GTG CAT CTC GAG					
D D V T P T K H A I V Q T L V H L E					
1761	1770	1779	1788	1797	1806
TTC CCC ACA AAG GTG GGC AAA GCC TGC TGC GTT CCC ACC AAA CTG AGT CCC ATC					
F P T K V G K A C C V P T K L S P I					
1815	1824	1833	1842	1851	1860
TCC ATC CTC TAC AAG GAT GAC ATG GGG GTG CCA ACC CTC AAG TAC CAC TAT GAG					
S I L Y K D D M G V P T L K Y H Y E					
1869	1878	1887	1903	1913	1923
GGG ATG AGT CTG GCT GAG TGT GGG TGT AGG TAGTCCCTGC AGCCACCCAG GGTGGGGATA					
G M S Y A E C G C R					

(428)

4/8

Figure 1D

1933	1943	1953	1963	1973	1983	1993
CAGGACATGG	AAGAGGTTCT	GGTACGGTCC	TGCATCCTCC	TGCGCATGGT	ATGCCTAAGT	TGATCAGAAA
2003	2013	2023	2033	2043	2053	2063
CCATCCTTGA	GAAGAAAAGG	AGTTAGTTGC	CCTTCTTGTC	TCTGGTGGGT	CCCTCTGCTG	AAGTGACAAT
2073	2083	2093	2103	2113	2123	2133
GACTGGGGTA	TGCGGGCCTG	TGGGCAGAGC	AGGAGACCCT	GGAAGGGTTA	GTGGGTAGAA	AGATGTCAAA
2143	2153	2163	2173	2183	2193	2203
AAGGAAGCTG	TGGGTAGATG	ACCTGCACTC	CAGTGATTAG	AAGTCCAGCC	TTACCTGTGA	GAGAGCTCCT
2213	2223	2233	2243	2253	2263	2273
GGCATCTAAG	AGAACTCTGC	TTCCTCATCA	TCCCCACCGA	CTTGTTCTTC	CTTGGGAGTG	TGTCCTCAGG
2283	2293	2303	2313	2323	2333	2343
GAGAACAGCA	TTGCTGTTCC	TGTGCCTCAA	GCTCCCAGCT	GACTCTCCTG	TGGCTCATAG	GACTGAATGG
2353	2363	2373	2383	2393	2403	2413
GGTGAGGAAG	AGCCTGATGC	CCTCTGGCAA	TCAGAGCCCG	AAGGACTTCA	AAACATCTGG	ACAACTCTCA
2423	2433	2443				
TTGACTGATG	CTCCAACATA	ATTTTTAAAA	AGAG			

578

Figure 2

10 20 30 40 50 60 70  
 CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCAGG CCGGAAGCTA GGTGAGTGTG  
 80 90 100 110 120 130 140  
 GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC  
 150 160 170 180 190 200 210  
 GATGGGATT CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG  
 220 230 240 250 260 270 280  
 CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC  
 290 300 310 320 330 340 350  
 GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG  
 360 370 380 390 400 (1)  
 CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT  
 MET Ile Pro  
 417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 477 492 507  
 AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 522 537 552 567  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 642 657 672  
 AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
 747 762 777  
 AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
 792 807 822 837  
 CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

SUBSTITUTE SHEET

6/8

Figure 2A

852 867 882 897  
 CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

912 927 942  
 GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957 972 987 1002  
 ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062 1077 1092 1107  
 GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1182 1197 1212  
 CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys

1287 1302 1317  
 CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGC  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg

1332(311) 1347 1362 1377  
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

1392 1407 1422 1437  
 GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497 1512 1527 1542  
 GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

SUBSTITUTE SHEET

2/2

Figure 2B

1557                      1572                      1587  
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 1602                      1617                      (408)                      1636                      1646                      1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG  
MET Val Val Glu Gly Cys Gly Cys Arg  
 1666                      1676                      1686                      1696                      1706                      1716                      1726  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC  
 1736                      1746                      1756                      1766                      1776                      1786                      1796  
 ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC  
 1806                      1816                      1826                      1836                      1846                      1856                      1866  
 ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA  
 1876                      1886                      1896                      1906                      1916                      1926                      1936  
 ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAACT  
 1946  
 CTAGAGTCGA CGGAATTC

SUBSTITUTE SHEET



p/8

Figure 3

TGA	ACA	AGA	GAG	TGC	TCA	AGA	AGC	TGT	CCA	AGG	ACG	GCT	CCA	CAG	AGG	48
*	Thr	Arg	Glu	Cys	Ser	Arg	Ser	Cys	Pro	Arg	Thr	Ala	Pro	Gln	Arg	
-41	-40					-35					-30					
CAG	GTG	AGA	GCA	GTC	ACG	AGG	AGG	ACA	CGG	ATG	GCG	CAC	GTG	GCT	GCG	96
Gln	Val	Arg	Ala	Val	Thr	Arg	Arg	Thr	Arg	Met	Ala	His	Val	Ala	Ala	
-25					-20				-15						-10	
GGG	TCG	ACT	TTA	GCC	AGG	CGG	AAA	AGG	AGC	GCC	GGG	GCT	GGC	AGC	CAC	144
Gly	Ser	Thr	Leu	Ala	Arg	Arg	Lys	Arg	Ser	Ala	Gly	Ala	Gly	Ser	His	
				-5					1				5			
TGT	CAA	AAG	ACC	TCC	CTG	CGG	GTA	AAC	TTC	GAG	GAC	ATC	GGC	TGG	GAC	192
Cys	Gln	Lys	Thr	Ser	Leu	Arg	Val	Asn	Phe	Glu	Asp	Ile	Gly	Trp	Asp	
		10					15					20				
AGC	TGG	ATC	ATT	GCA	CCC	AAG	GAG	TAT	GAA	GCC	TAC	GAG	TGT	AAG	GGC	240
Ser	Trp	Ile	Ile	Ala	Pro	Lys	Glu	Tyr	Glu	Ala	Tyr	Glu	Cys	Lys	Gly	
	25					30					35					
GGC	TGC	TTC	TTC	CCC	TTG	GCT	GAC	GAT	GTG	ACG	CCG	ACG	AAA	CAC	GCT	288
Gly	Cys	Phe	Phe	Pro	Leu	Ala	Asp	Asp	Val	Thr	Pro	Thr	Lys	His	Ala	
40					45				50						55	
ATC	GTG	CAG	ACC	CTG	GTG	CAT	CTC	AAG	TTC	CCC	ACA	AAG	GTG	GGC	AAG	336
Ile	Val	Gln	Thr	Leu	Val	His	Leu	Lys	Phe	Pro	Thr	Lys	Val	Gly	Lys	
				60					65					70		
GCC	TGC	TGT	GTG	CCC	ACC	AAA	CTG	AGC	CCC	ATC	TCC	GTC	CTC	TAC	AAG	384
Ala	Cys	Cys	Val	Pro	Thr	Lys	Leu	Ser	Pro	Ile	Ser	Val	Leu	Tyr	Lys	
			75					80					85			
GAT	GAC	ATG	GGG	GTG	CCC	ACC	CTC	AAG	TAC	CAT	TAC	GAG	GGC	ATG	AGC	432
Asp	Asp	Met	Gly	Val	Pro	Thr	Leu	Lys	Tyr	His	Tyr	Glu	Gly	Met	Ser	
		90					95					100				
GTG	GCA	GAG	TGT	GGG	TGC	AGG	TAGTATCTGCTGCGGG								470	
Val	Ala	Glu	Cys	Gly	Cys	Arg										
	105					110										

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/05374

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC - Int.Cl. 5 C12N15/12; C12P21/02; A61K37/02		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	C07K ; C12N ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	WO,A,9 011 366 (GENETICS INSTITUTE, INC.) 4 October 1990 cited in the application see the whole document ---	1-18
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, no. 24, December 1990, WASHINGTON US pages 9843 - 9847 CELESTE, A.J. ET AL. 'Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone' see the whole document --- -/--	1-18
<p><sup>10</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 05 OCTOBER 1992		Date of Mailing of this International Search Report 19. 10. 92
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer ANDRES S.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,A	WO,A,9 118 098 (GENETICS INSTITUTE, INC.) 28 November 1991 cited in the application see the whole document -----	1-18

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 16, 18 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged affects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9205374  
SA 61850

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 05/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9011366	04-10-90	US-A- 5106748	21-04-92
		AU-A- 5357790	22-10-90
		CA-A- 2030518	29-09-90
		EP-A- 0429570	05-06-91
		JP-T- 3505098	07-11-91
-----			
WO-A-9118098	28-11-91	None	
-----			

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☒ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**